REMARKS

Claims 32 to 36 are pending in the application.

1.2 - Drawings

The objections to the Figures for the reasons indicated in point 1.2 of the Office Action are respectfully traversed and reconsideration thereof is respectively requested on the following grounds.

1. The data of Figs. 1 to 8 and 10-16 are pertinent to SEQ ID NO:2.

Figs. 1 to 8, 10-14 and 16 describe experiments performed on demethylase prepared from human non-small cell lung carcinoma A549. Fig. 11 shows the results of expression of transfected dMTase cDNA in human embryonal kidney cells (page 39, line 26 to page 40, line 9). Fig. 13a-d indicate which cells are pertinent (see also page 41, line 14 to page 42, line 9). Fig. 15 shows results of induced expression of DNA demethylase in the antisense orientation transfected into HEK 293 cells (page 43, lines 3-17).

2. Fig. 8c illustrates the expression of human dMTase1, SEQ ID NO:2, in human tissues from a normal human person.

3. Fig. 10b does not show that the efficiency of cytosine demethylation by dMTase is increased with imidazole. The experiment describes elution of His-tagged dMTase from a nickel resin column with imidazole (page 21, lines 5-21). The imidazole concentrations shown are not the concentration of imidazole in the reaction but the concentrations used to elute dMTase1 (SEQ ID NO:2). As described on page 21, lines 5-21, after elution, the eluate was dialyzed. There is no imidazole in the reaction.

4. As discussed for item 3 above, the concentration of imidazole indicated in Fig. 11 is the concentration used to elute the protein from the nickel resin (see page 39, lines 25-31).

5. One of skill in the art would understand upon reading the paragraph bridging pages 39 and 40 that "transforms methylated cytosine to cytosine in a protein dependent manner" means that transiently expressed dMTase demethylates DNA (page 39, line 24), and thus methylated cytosine would be converted to (non-methylated) cytosine and that the reaction depends on the presence of dMTase in the reaction since degradation of dMTase by proteases abolishes the reaction.

6. Page 10, line 19 of the application has been amended to correct the term "tumorigenesis" with the phrase "growth on soft agar of colonies of tumor cells" as suggested by the Examiner. Support for this amendment can be found in Fig. 14c.

7. Page 10, line 21 of the application has been amended to correct the phrase "inhibition of tumorigenesis" with the phrase "reduced colony formation of HEK 293 cells" as suggested by the Examiner. Support for this amendment can be found on page 43, lines 3 to 17 of the application. The antisense nucleic acid sequence for demethylase dMTase1 was used.

The comments and amendments to the specification, as discussed above, are believed to overcome the Examiner's objections.

2.2 - 35 USC § 112, second paragraph

The rejections of claims 32-38 under 35 USC § 112, second paragraph, is respectfully traversed and reconsideration thereof is respectfully requested on the following grounds.

Claims 37 and 38 have been previously cancelled.

Claim 36 has been amended to replace the phrase "a change of" with the word "altering", antecedent basis for which is found in claim 32.

Claim 35 has been amended to replace the phrase "an imidazole derivative" with the phrase "imidazole and derivatives" as suggested by the Examiner.

Claim 32 has been amended as a Markush claim and to remove the phrase "or a homologue thereof" and add "SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8".

The amendments as presented above are believed to overcome the Examiner's rejections to claims 32 to 38 under 35 USC § 112, second paragraph.

2.2 - 35 USC, § 112, first paragraph

The rejection of claims 32 to 38 under 35 USC § 112, first paragraph for failing to comply with the written description requirement, is respectfully traversed and reconsideration thereof is respectfully requested on the following grounds.

Claims 37 and 38 have been previously cancelled.

Claim 32 is directed to a method of inhibition of tumorigenesis. In support of the fact that the Applicants were in possession of the claimed invention at the time the application was filed, Applicants present the articles of Slack *et al.* (2002) J. Gene Med., 4:381-389, as Exhibit A, Ivanov *et al.* (2003) J. Gene Med., 5:893-899, as Exhibit B, and Campbell *et al.* (2004) Carcinogenesis, 25(4):499-507, as Exhibit C, which show that inhibition of demethylase (SEQ ID NO:2) by an antisense molecule inhibits tumorigenesis *in vivo*. All of the above published data came as a direct result of the present application.

The Examiner's comment that there is a lack of written description of alteration of any methylation pattern in any patient, is traversed and reconsideration thereof is respectfully requested on the following grounds. Claim 32 has also been amended to indicate that the method of inhibition of tumorigenesis comprises the step of administering an effective amount of an antagonist or inhibitor of DNA demethylase, thereby altering a methylation pattern in DNA.

The present application discusses different assays to determine DNA demethylase activity, for example page 26, lines 3-14, page 27, line 15 to page 28, line 10, page 31, line 5 to page 32, line 25 and page 39, line 24 to page 40, line 9. Such assays show that methylated DNA is demethylated in the presence of DNA demethylase (dMTase). Figure 6, described on page 31, line 5 to page 32, line 25, shows that the state of methylation of plasmid DNA was determined using methylation sensitive enzymes. Page 43, lines 18-29 of the application for example shows that DNA dMTase activity is inhibited by imidazole. Such

inhibition of dMTase activity therefore alters the methylation pattern of a DNA in the presence of dMTase. Thus, the Applicant believes there is not a lack of written description for alteration of a methylation pattern of DNA and that one of skill in the art would realize that the inventors had possession of the claimed invention at the time the application was filed.

As discussed above, amended claim 32 is now directed to a method of inhibition of tumorigenesis wherein production of DNA demethylase in increased in comparison with that of a non-tumor cell. Claim 32 is no longer also directed to a method of altering a methylation pattern in DNA. The arguments and amendments discussed above are believed to overcome the Examiner's rejection of claims 32-36 for lack of written description of tumorigenesis phenomenon to be inhibited and of alteration of any methylation pattern in a patient by use of an inhibitor of DNA demethylase.

The Examiner's rejection of the terms "antagonist" and "inhibitor" is respectfully traversed. Applicant believes that it has sufficiently described the concept of DNA demethylase antagonists and inhibitors generically, which conclusively demonstrates that the Applicant was in possession of the generic concept at the time the application was filed. The Applicant has described a broad range of specific examples of DNA demethylase inhibitors which show that DNA demethylase inhibitors are broadly applicable in the invention. There is no evidence which would raise doubt as to the operability and effectiveness of any DNA demethylase inhibitor in the invention. In support of this, Applicants attach herewith additional data, in a Rule 132 Declaration, as Exhibit D, from

Dr. Moshe Szyf, a co-inventor of the present invention, showing that methyl thio-adenosine (MTA) is also an inhibitor of demethylase, including a copy of Detich *et al.* (2003) J. Biol. Chem. 278:20812-20820, enclosed therein, which shows that the methyl donor S-adenosylmethionine inhibits active demethylation of DNA. Applicant wishes to express that such additional inhibitors could be determined by routine experimental techniques known to one of skill in the art and no undue burden would be placed on one of skill in the art to determine additional inhibitors. Further, the law does not require the Applicant to disclose every possible DNA demethylase inhibitor in order to claim the invention generically but, only various examples, which was done for example on page 43, lines 18-29 of the application. Reconsideration of this rejection by the Examiner is therefore respectfully requested.

The amendment of claim 32 to remove the phrase "or a homologue thereof", as discussed above, is believed to render the Examiner's rejection of claims 32-36 on page 8 of the Office Action for use of this term moot.

The rejection of claim 36 for being directed to a method of inhibition of tumorigenesis and altering methylation of DNA, wherein alteration of the methylation pattern activates a silent gene, is respectfully traversed and reconsideration thereof is respectfully requested on the following grounds.

Claim 36 has been amended to be directed to a method wherein altering the methylation pattern silences a gene. As known in the prior art, for example see the review by Szyf (2003, Drug Resistance Updates, section 5), as Exhibit E: "silencing of tumor suppressor genes by hypermethylation is well documented";

and page 1, lines 9-15 of the present application: altering a methylation pattern causes differential gene expression. One of skill in the art would realize and would be able to soundly predict based on the teaching of the present disclosure that inhibition of demethylase would promote methylation of a gene, which in turn would silence the gene. Further, no undue burden would be placed on one of skill in the art to arrive at amended claim 36. In further support of this amendment, Applicants attach herewith additional experimental data, in the aforementioned Rule 132 Declaration (Exhibit D), showing that anti-sense inhibition of DNA demethylase (SEQ ID NO:1) promotes inhibition (silencing) and remethylation of uPA, including a copy of Herman et al. (1996) Proc. Natl. Acad. Sci. USA, 93:9821-9826, enclosed therein.

The amendment to claim 36 and the comments presented above are believed to overcome the Examiner's rejection of claim 36, for allegedly not convincing one of skill in the art that the Applicants were in possession of the claimed invention when the application was filed.

2.2.2 – Scope of Enablement

The Examiner's rejection of claims 32-36 under 35 USC § 112, first paragraph for allegedly not enabling any person skilled in the art to make and use the invention commensurate in scope with the claims is respectfully traversed and reconsideration thereof is respectfully requested on the following grounds.

In support of enablement of the claimed invention, Applicants present the articles of Slack *et al.* (2002) J. Gene Med., 4:381-389, as Exhibit A, Ivanov *et al.* (2003) J. Gene Med., 5:893-899, as Exhibit B, and Campbell *et al.* (2004) Carcinogenesis, 25(4):499-507, as Exhibit C, as discussed above, which show that inhibition of demethylase (SEQ ID NO:2) by an antisense molecule inhibits tumorigenesis *in vivo*. All of the above published data came as a direct result of the present application. Further, claim 32 has been amended to be directed to a method of inhibition of tumorigenesis wherein production of DNA demethylase is increased in comparison with that of a non-tumor cell. Support for this amendment is found on page 5, lines 8-11, page 8, line 20-21 and page 41, line 14 to page 42, line 9. In addition, as discussed above, Applicant believes that the claims should not be limited in its use of an antagonist or inhibitor. Thus, Applicant believes that one of skill in the art would not require undue experimentation to make the claimed invention.

The amendments and arguments presented above are believed to overcome the Examiner's rejection of claim 32 for not enabling any person skilled in the art to make and use the invention commensurate in scope with the claims. Claims 33-36, dependent on claim 32, are also therefore believed to overcome the Examiner's rejection.

2.2.3 – Lack of Enablement

The Examiner's rejection of claims 32-36 under 35 USC § 112, first paragraph, for allegedly being non-enabling, is respectfully traversed and reconsideration thereof is respectfully requested on the following grounds.

As discussed above, claim 32 has been amended to be directed to a method of inhibition of tumorigenesis wherein production of DNA demethylase is increased in comparison with that of a non-tumor cell comprising the step of administering an effective amount of an antagonist or inhibitor of DNA demethylase, thereby altering a methylation pattern in DNA. One of skill in the art would realize that the phrase "a methylation pattern in DNA" is commonly known and understood in the art. For example, page 1 of the application indicates that the pattern of methylation is fashioned by a sequence of methylation and demethylation events of the dinucleotide sequence CpG. Further, page 31, line 5 to page 32, line 25 for example, shows a method to measure and visualize the pattern of DNA methylation of DNA and how to alter it with demethylase.

The amendments and arguments presented above are believed to overcome the Examiner's rejection of claim 32 for being not enabling. Claims 33-36, dependent on claim 32, are also therefore believed to overcome the Examiner's rejection.

The Applicants submit that no new matter has been added by the present amendments.

It is submitted, therefore, that the claims are in condition for allowance. Reconsideration of the Examiner's rejections is respectfully requested. Allowance of claims 32-36 at an early date is solicited.

If there are any questions regarding this amendment or the application in general, a telephone call to the undersigned would be appreciated since this should expedite the prosecution of the application for all concerned.

If necessary to effect a timely response, this paper should be considered as a petition for an Extension of Time sufficient to effect a timely response, and please charge any deficiency in fees or credit any overpayments to Deposit Account No. 05-1323 (Docket #03863048896).

Respectfully submitted,

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